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ADVANCES IN MECHANICALLY SCANNED ACOUSTIC MICROSCOPY,(U)
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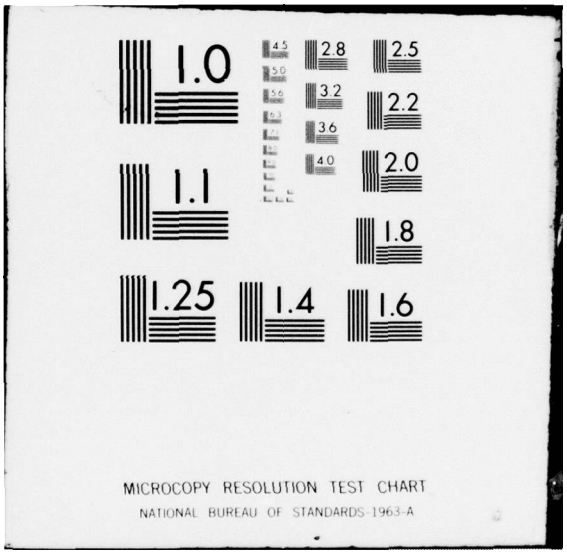
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by

R. A. Lemons and C. F. Quate

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ABSTRACT

A scanning acoustic microscope, using the design we have previously described, can now be routinely operated at a frequency of 1 GHz with a corresponding resolution of nearly 1 μm. Operating in transmission a number of biological samples have been imaged. With this high resolution, variations in the elastic properties within individual cells can be seen. Moreover, clear distinctions have been demonstrated between the acoustic images and corresponding optical micrographs. Several alternative imaging modes have also been demonstrated with this instrument. One of these is a phase contrast technique in which the phase of the output signal is compared directly to the phase of the input signal. Another mode is reflection imaging which allows specimens on thick substrates to be viewed. This technique has been applied profitably to an investigation of integrated circuits.

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R. A. Lemons and C. F. Quate

I. INTRODUCTION

The development of the mechanically scanned acoustic microscope has been progressing in steps of increasing operating frequency. The microscope which is described in this paper is a third generation instrument having been preceded first by a 160 MHz model[1] and then a 400 MHz model.[2] The present instrument is generally operated in the frequency range of 600 to 1000 MHz. At the high end of this range the acoustic microscope achieves a resolution of approximately 1 μm.

The design of this high frequency microscope is basically the same as that of its predecessors. A plane acoustic wave is generated in a sapphire crystal by a piezoelectric transducer on one of the crystal faces. In this case the transducer is an r.f. sputtered ZnO film with a 75% bandwidth centered at 750 MHz. At the end of the crystal opposite the transducer a single surface lens focuses the collimated beam into a water cell as shown in Fig. 1. The large difference between the acoustic velocity in sapphire and in water is relied upon to reduce the spherical aberration of this simple lens to a size which is well below the diffraction limitations.[2] In the absence of spherical aberration a lens with a very small f number can be used. This enables the acoustic beam to be focused to a waist a fraction of an acoustic wavelength in diameter.

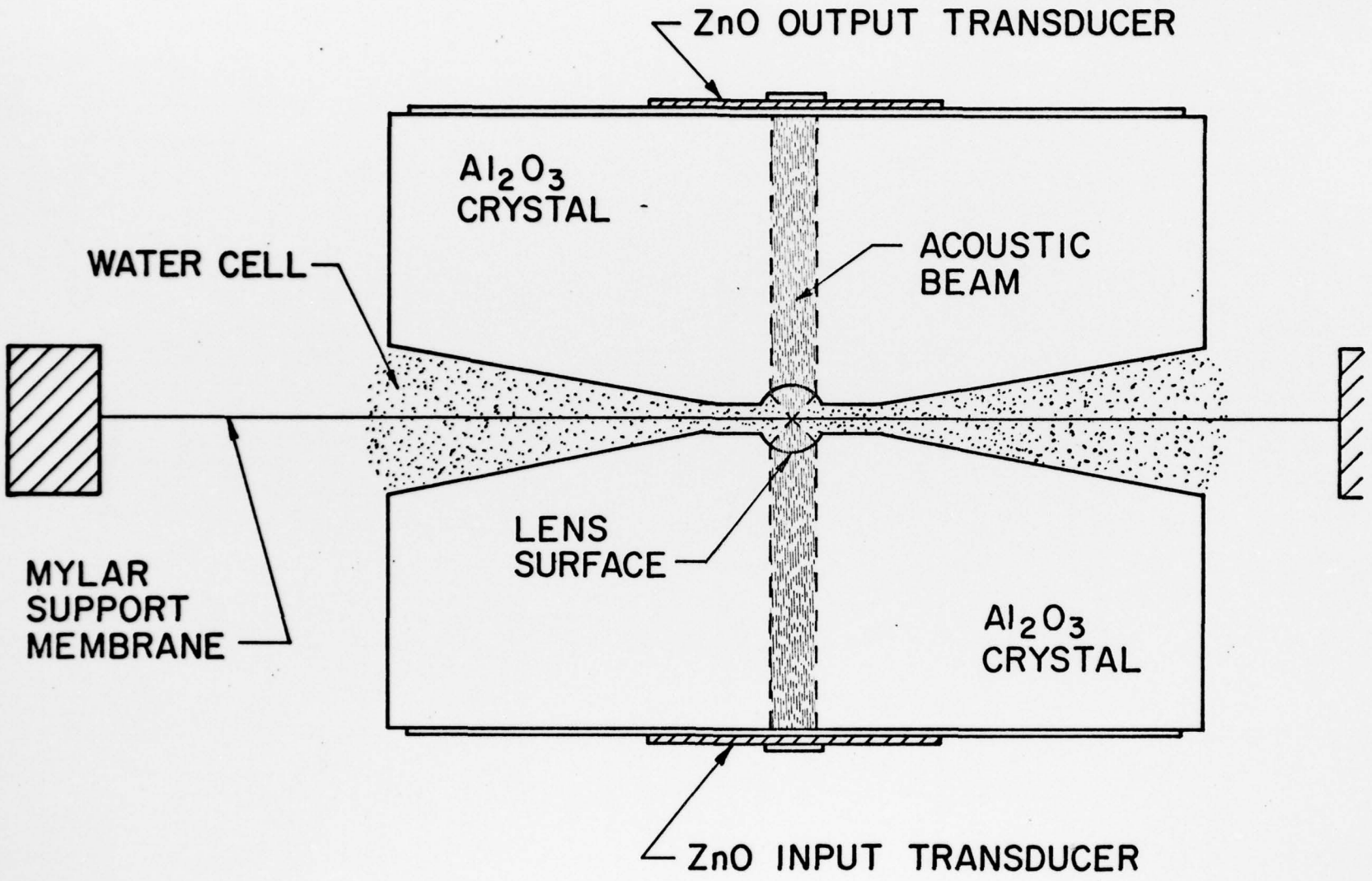

Fig. 1 Schematic showing high frequency lens design.

The obstacle that is encountered in achieving high resolution with this system is that the acoustic attenuation (measured in decibels) through the liquid cell increases as the square of the acoustic frequency. The liquid is, of course, necessary to allow the object to be scanned relative to the focus of the lenses. To compensate for the extremely high acoustic attenuation found at 1 GHz, it was necessary to fabricate very tiny lenses. In the present system the lenses have a focal length of 0.15 mm and an f number of 0.75.

The object to be imaged is supported at the focal plane of this lens by a 2 μm thick mylar membrane. To achieve a two-dimensional acoustic image of this object, a mechanical system is used to scan the object in a raster pattern. This mechanical scanning is accomplished by connecting the object to the cone of a loudspeaker. While the loudspeaker scans the object rapidly in one dimension the entire assembly of loudspeaker and object is moved slowly in the orthogonal dimension. The physical motion of the object is synchronized with the electron beam of a cathode-ray display on which the image is viewed. Since the objects of interest are small and light, the scanning can be done rapidly enough to complete a frame in about one second.

II. TRANSMISSION ACOUSTIC MICROSCOPY

Referring again to Fig. 1, the fraction of acoustic power which is transmitted by a given point on the object is collected by a second lens which is confocal to the first. In this geometry the beam is recollimated and detected with a second piezoelectric transducer. It is the signal

generated by this detector which is used to modulate the intensity of the display to produce a visual image.

A photograph of the mechanical components of the acoustic microscope is shown in Fig. 2. The two lens mounts used in transmission imaging are held by the vertical supports visible in the center of the photograph. Here the lenses have been pulled apart to give a better view of the scan drive. In operation the lenses are separated by approximately 200 μm. The water cell defined by these lenses is maintained by the surface tension. The object to be imaged is mounted on the ring that can be seen just above the lenses. This ring is connected by a rod to the loudspeaker behind. The hydraulic system which moves the speaker assembly is obscured from view. Since it is necessary to align the two lenses with great precision the mechanical support for the left-hand lens allows it to be moved accurately over micrometer distances.

The most interesting samples imaged in the transmission mode have been of biological origin. One type of sample which we frequently image as a test object is a simple blood smear. Fresh blood is smeared onto the mylar support membrane and is then fixed with methanol. In a smear from a normal individual the erythrocytes, or red blood corpuscles, comprise the vast majority of cells. These cells are between 6 and 8 μm in diameter and are composed largely of hemoglobin. As an illustration of this type of preparation Fig. 3 shows a comparison of the acoustic and optical images of these cells. In this case the acoustic image was taken at 900 MHz. The optical image was made with an American Optical Microstar 10 with a 40X NA/66 objective; the condenser was adjusted for optimum resolution and contrast.

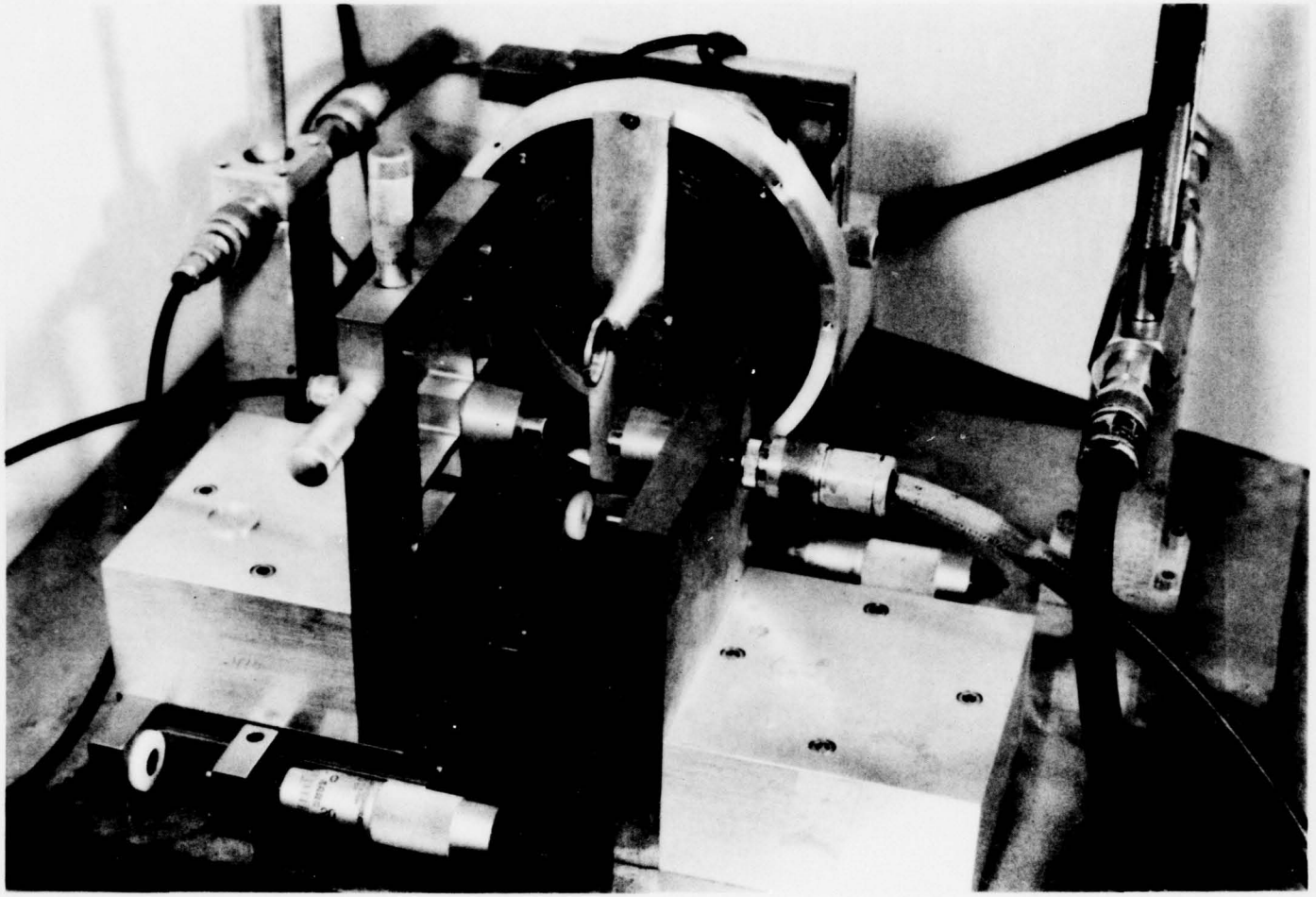

Fig. 2 Photograph of the acoustic microscope's mechanical components.

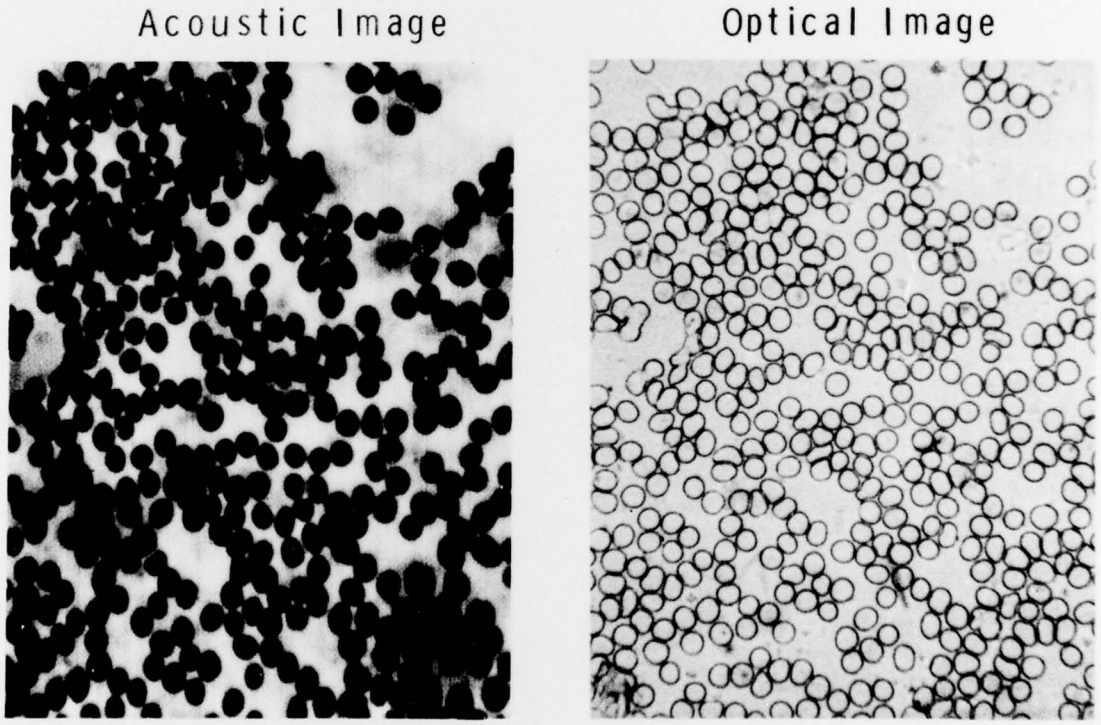

Fig. 3 Comparison of the acoustic and optical images of human red blood cells. The acoustic image was made with a frequency of 900 MHz.

It can be seen that the shape of the cells in the acoustic image corresponds closely with that of its optical counterpart. The most striking feature, however, is the superb contrast seen in the acoustic image. The optical image, by comparison, exhibits low contrast which is to be expected since the sample is unstained. Many biological samples are nearly transparent to optical waves unless they are stained.

More interesting acoustic images are obtained from specimens containing a variety of cell types. As an illustration of such a specimen, Fig. 4 shows the acoustic image of a section of rat kidney tissue. The sample was prepared by microtoming a 5 μm thick section from a block of fixed and embedded tissue. This section was then mounted on the mylar support membrane. The acoustic image shows the morphology of the tissue quite clearly. The section was taken from the deep cortical area. A number of renal tubules can be seen both in cross section and in longitudinal section. Each of these tubules is composed of a single layer of cells. In many cases the nucleus within each cell is clearly resolved. Without the addition of stains, little of this information could be obtained with a light microscope.

Since the acoustic microscope is sensitive to the elastic properties of the specimen, it is important to ascertain which features of a tissue are distinguished on this basis. Figure 5 shows a comparison of the acoustic and optical images of a section from a malignant breast tumor. After the acoustic image was made, the specimen was stained with hematoxylin-eosin. This stain provides the contrast necessary to take the optical micrograph. The prominent feature in both of these images is the duct-like structure at the left. A group of neoplastic cells have differentiated to form this

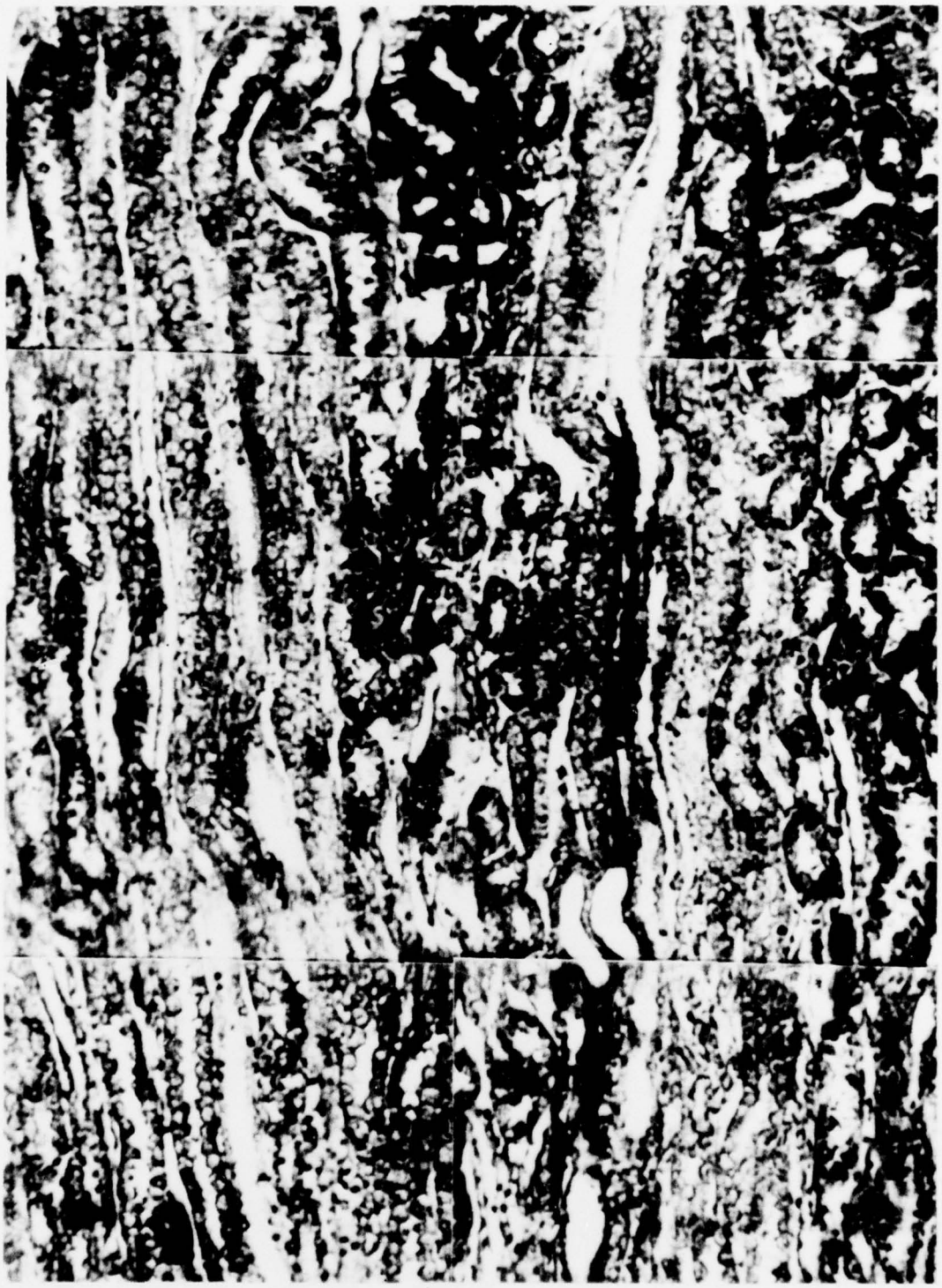

Fig. 4 Acoustic image (600 MHz) of a section of rat kidney. The sample is unstained.

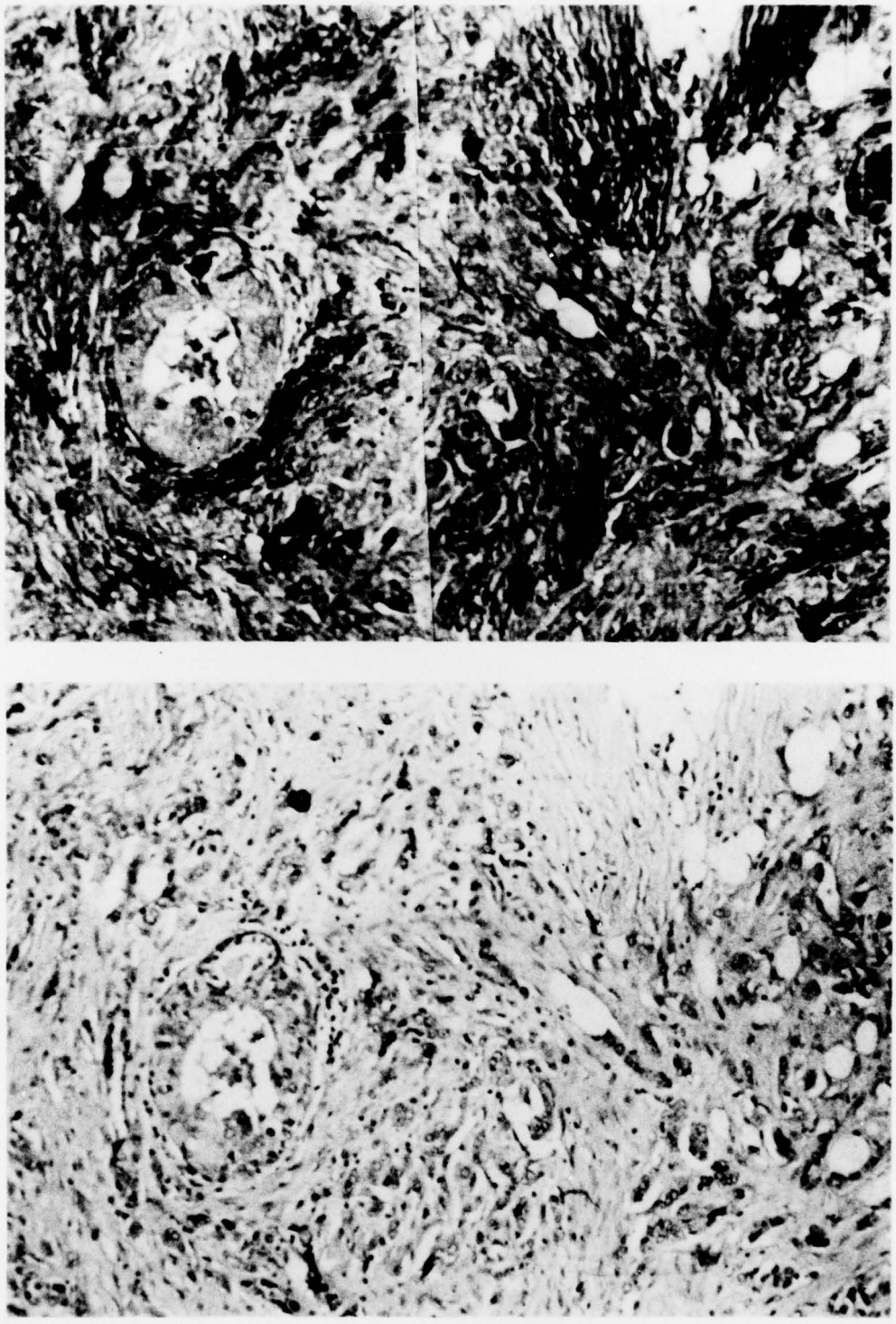

Fig. 5 Top - Acoustic micrograph (600 MHz) of a malignant tumor of the human breast (unstained).
Bottom - Optical micrograph of the same area stained with hematoxylin-eosin.

duct in a fashion characteristic of this particular cancer. Interesting distinctions can be seen between the acoustic and the optical images. For example, the fibrous connective tissue in the upper right corner is made apparent by its large absorption. Only subtle differences between this area and adjacent ones can be seen in the light micrograph. There are other small nodules with very large acoustic absorption which are not distinguished in the optical image. The body of information of this kind which we have accumulated leads us to believe that there may be important diagnostic applications for the acoustic microscope.

Recently we have done some experiments in the transmission geometry which indicate that information concerning the nonlinear elastic properties of the sample can be obtained.[3] Owing to the high convergence provided by the acoustic lens, sufficient power densities can be produced at the focus to generate a detectable second harmonic signal. The images which have been obtained by modulating the display with this second harmonic signal show marked differences when compared with linear absorption images. We believe that a gamut of new information may be uncovered by using this nonlinear technique to probe the sample.

III. PHASE CONTRAST ACOUSTIC MICROSCOPY

The design of the scanning acoustic microscope makes phase contrast imaging particularly easy to achieve. By comparing the phase of the output from the microscope with a constant phase reference signal the acoustic delay through each point on the sample can be measured. Displaying this information results in a phase contrast image. In our present system this

has been accomplished by using a directional coupler to split off part of the input power to provide a reference. This reference was then amplified to a suitable level and mixed with the output signal from the microscope. The resulting base band signal was then amplified and applied to the intensity modulation circuit of the display.

An example of the phase contrast images generated in this way are shown in Fig. 6. The sample was a section of human lung tissue. The region that is imaged shows the folded epithelium of a small bronchiole. The photograph at the left of this figure is a standard transmission image of the area. In the center the 'bright' phase contrast image is shown while the 'dark' phase contrast image is at the right. The appearance of the image can be varied uniformly between these two extremes by introducing a variable phase into either the reference circuit or the microscope circuit. In this case the separation between the acoustic lenses was increased by approximately half of an acoustic wavelength in going from one image to the other. Some interesting comparisons can be made between these phase contrast images and the transmission image. However, the amplitude modulation is so strong that much of the phase information is obscured. The real utility of the acoustic phase contrast technique will be on samples which show a low uniform acoustic absorption.

IV. ACOUSTIC REFLECTION IMAGING

For certain samples it is advantageous to form an image with reflected acoustic energy rather than transmitted energy. This has been accomplished in the present scanning system by using a single lens element. In this case

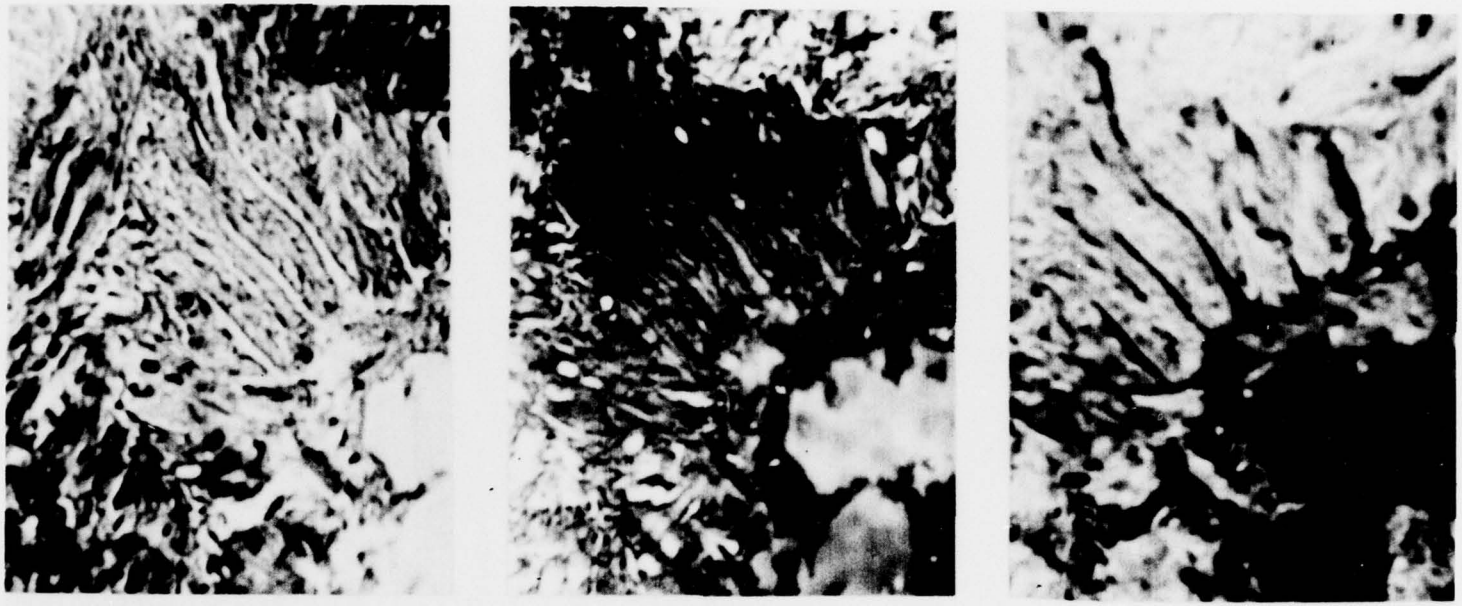

Fig. 6 (A) Absorption image. (B) 'Bright' phase contrast image. (C) 'Dark' phase contrast image. These acoustic images show the epithelium of a small bronchiole in the human lung.

the one transducer acts both as the generator and the detector of the acoustic signal. The generated acoustic wave is focused into the water by the lens surface just as in the transmission mode. A portion of this energy will be reflected by a specimen placed at the lens focus. This same lens will recollimate the reflected wave in the sapphire crystal. The particular technique we have employed operates the system in a CW fashion. In order to separate the input electrical signal from the reflected signal, a microwave circulator is inserted into the system. The signal reflected from the specimen is, however, only a small part of the total reflected signal. To isolate the portion of interest, the scanning of the specimen is used to great advantage. Because of the scanning, information reflected from the sample appears as a time varying modulation of the total signal. Thus, a filter can be employed in the final detected output to separate the image information from the background. We have applied this acoustic reflection technique to an investigation of integrated circuits. The silicon substrate for an integrated circuit is usually too thick to be accommodated in the transmission system. The interesting details are, however, near the surface. This makes the integrated circuit particularly suitable for reflection imaging. The general features of the acoustic reflection images produced with this system as well as the potential applications of acoustic imaging to integrated circuit testing has been described in another publication.[4]

V. CONCLUSIONS

The mechanically scanned acoustic microscope is an instrument of rather simple design. Because of this it has been possible to achieve 1 μm resolution. With this resolution the acoustic microscope now becomes a practical tool which can complement and extend the information available from light microscopy. The same basic instrument can be used not only in transmission but in phase contrast and reflection modes as well. The ability to map elastic properties on this scale should give us new access to significant structure in the microscopic world.

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